Table I. Reaction of C_8K with Aromatic Acid Esters

ester	product	yield (THF), %	yield (DME), %
6a	4	30	30
6b	4	30	30
7a	9	26	40
7b	9	41	38
8	10	30	30
11	4	30	13
	12	16	27
	13	30	19
	14	5	4

that treatment of fluorenone with C_8K yielded only **12** and **13**.

The bimolecular reactions of **6–8** involve two steps: (I) formation of benzil from the ester, following the classical acyloin condensation pattern; (II) the conversion of benzil to phenanthrenequinone[3] (Figure 2). The combination of these two synthetic steps in one reaction and the facile conversion of very simple and inexpensive starting compounds into complex products make the reaction of aromatic acid esters with C_8K an attractive and valuable synthetic tool. Although the overall yields of the reaction are not high, the efficiency of the coupling step is much better than in the classical acyloin condensation. Furthermore, the succeeding ring closure provides a new entry to the preparation of phenanthrenequinone derivatives from readily available single ring compounds.

Our results are also interesting from the mechanistic point of view. In all the various reactions of C_8K that lead to phenanthrenequinone formation, the mechanistic pathway involves the conversion of $\mathbf{3}^{2-}$ to **4**. In the case of the aromatic esters, it seems that dianion $\mathbf{3}^{2-}$ is formed by reduction of benzil (**3**) with excess C_8K, the benzil having been formed by cleavage of two methoxy groups from the biradical coupling product (Figure 2).

These results shed light on the controversial mechanism of the acyloin reaction,[5,6] proving that benzil *is* an intermediate in this condensation. The fact that a dianion of an α-diketone is essential to the further ring-closure process on the one hand and the formation of the diketone **4** from the diphenic acid ester on the other may serve as evidence for the existence of benzil as a key intermediate in the acyloin condensation sequence.

Experimental Section

General Methods. All C_8K preparations and reactions were carried out in flame-dried glassware under argon atmosphere. Tetrahydrofuran was dried over potassium metal and distilled prior to use; dimethoxyethane was dried by distillation over lithium aluminum hydride. NMR spectra were recorded on Bruker WH-300 pulsed FT spectrometer operating at 300.133 MHz. Chemical shifts are expressed in ppm downfield from Me_4Si. Mass spectra were determined on Varian MAT 311 (70 eV). A Perkin-Elmer 157 G spectrophotometer was used for IR spectra. Melting points (°C) are uncorrected.

Preparation of Starting Esters 6–8 and 11. All the esters were prepared from the appropriate acids and alcohols according to the literature procedure.[7]

Preparation of C_8K.[8] Graphite powder (2.4 g, BDH, synthetic) was placed in a 100-mL argon-flushed and flame-dried three-necked round-bottomed flask at 150 °C, magnetically stirred and kept under argon atmosphere. After 15 min, 1 g (25 mmol) of clean potassium metal was added in slices. The stirring at 150 °C was continued until the bronze-colored C_8K was formed. The reagent was cooled to room temperature and kept under argon.

3,6-Diisopropylphenanthrenequinone (10). Freshly prepared C_8K (25 mmol), placed in the apparatus described above, was covered with 50 mL of dry solvent (dimethoxyethane or tetrahydrofuran) and kept at 25 °C under argon atmosphere. Magnetic stirring was started, and a solution of 710 mg (4 mmol) of methyl *p*-isopropylbenzoate (**8**) in 50 mL of solvent was dropped into the reaction mixture over 20 min. The reaction was monitored by TLC (SiO_2; methylene chloride/petroleum ether, 1:2). After **8** had disappeared, the mixture was stirred for 1 h and then cooled to 0 °C, and 10 mL of water was added to the solution.[9] The reaction mixture was filtered through a fritted glass funnel, and the filter cake was washed with two 25-mL portions of ether. The combined filtrate was washed twice with water (20 mL), the organic phase was dried over magnesium sulfate and filtered, and the solvent was removed by evaporation. The crude product (400 mg) was then purified on a chromatographic preparative plate (SiO_2; methylene chloride/petroleum ether 1:2). Crystallization from methanol gave 175 mg of **10** (30% yield): mp 155–157 °C; ^1H NMR ($CDCl_3$) 8.1 (d, 1 H, J = 8 Hz), 7.82 (s, 1 H), 7.27 (d, 1 H, J = 8 Hz), 3.11 (sept, 1 H, J = 7 Hz), 1.34 (d, 6 H, J = 7 Hz); IR 1672, 1600, 1455, 1376, 1282, 1060, 830 cm^{-1}; MS, m/e 292.

Anal. Calcd for $C_{20}H_{20}O_2$: C, 82.21; H, 6.89. Found: C, 82.41; H, 6.99.

Reaction of C_8K with Aromatic Esters: General Procedure. The procedure described above was applied to all aromatic acid esters and to diphenic acid ester. In a typical experiment the reaction time was 90–120 min. The melting points and spectra of the products were identical with the literature data. Melting point values of these products[10] are as follows: **4**, 206–208 °C; **9**, 113–116 °C; **12**, 114–116 °C, **13**, 152–153 °C; **14**, 81–83 °C.

Reaction of C_8K with Fluorenone. Freshly prepared C_8K (25 mmol) was covered by 50 mL of dry dimethoxyethane and kept at room temperature under argon atmosphere. Fluorenone (360 mg, 2 mmol) in 20 mL of dimethoxyethane was added, and the reaction mixture was magnetically stirred for 1 h. The reaction was worked up as described above. After chromatographic separation (SiO_2 preparative plate; methylene chloride/petroleum ether, 1:4) fluorene (150 mg; 0.94 mmol; 47%), mp 114–117 °C (lit. mp 117 °C) and flurenol (185 mg; 1.03 mmol; 25%), mp 151–153 °C (lit. mp 154 °C) were obtained. The products' spectra were identical with those of authentic samples.

Acknowledgment. Financial assistance from the Basic Research Foundation administered by the Israel Academy of Sciences and Humanities is gratefully acknowledged.

Registry No. **4**, 84-11-7; **6a**, 93-58-3; **6b**, 93-89-0; **7a**, 99-75-2; **7b**, 94-08-6; **8**, 20185-55-1; **9**, 60566-01-0; **10**, 108744-18-9; **11**, 5807-64-7; **12**, 86-73-7; **13**, 1689-64-1; **14**, 486-25-9; C_8K, 12081-88-8.

(6) An alternative mechanism has been suggested by: *Bloomfield*, J. J.; Owsley, D. C.; Einsworth, C.; Robertson, R. E. *J. Org. Chem.* **1975**, *40*, 893.

(7) Vogel, A. I. *Practical Organic Chemistry*, 3rd ed.; Longmans: New York, 1964; p 781.

(8) This procedure is modified, see: Lalancette, J. M.; Rollin, G.; Dumas, P. *Can. J. Chem.* **1972**, *50*, 3058.

(9) Addition of water to the reaction mixture at this stage does not involve any violent reaction.

(10) Literature mp: **4**, 208–210 °C; **9**, 212–213 °C; **12**, 117 °C; **13**, 153 °C; **14**, 83 °C. From: Heilbron, I. *Dictionary of Organic Compounds*, 5th ed.; Chapman and Hall: New York, 1982.

Synthesis of 5′-Carboxy-*N*′-nitrosonornicotine and 5′-([^{14}C]Carboxy)-*N*′-nitrosonornicotine[1]

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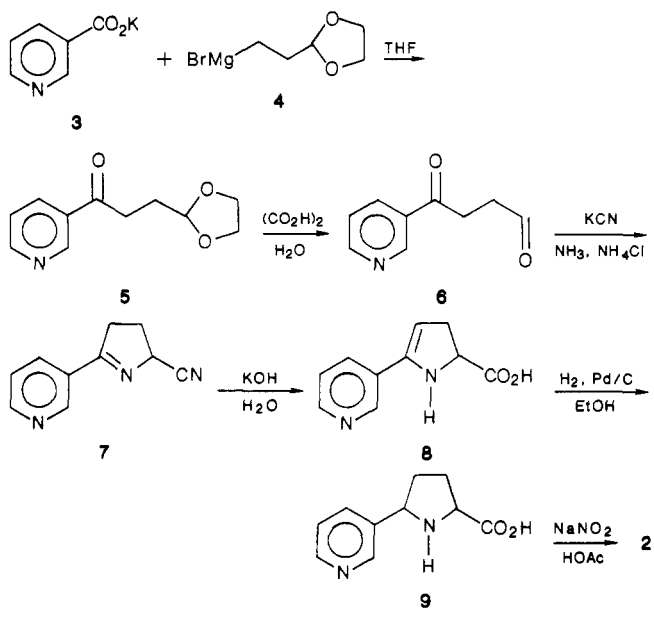
Received January 12, 1987

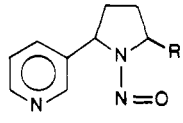
N′-Nitrosonornicotine (**1**) and the related nitrosamines that are formed from the tobacco alkaloids are considered to be among the most important compounds responsible

(1) This study was supported by Grant CA-35607 from the National Cancer Institute. This paper is dedicated to the memory of Aziz Abbaspour who passed away April 7, 1987.

Scheme I



for cancer induction by tobacco products.[2-4] 5′-Carboxy-*N*′-nitrosonornicotine (**2**) could be employed as an indicator of **1** produced endogenously from nicotine and nitrite.



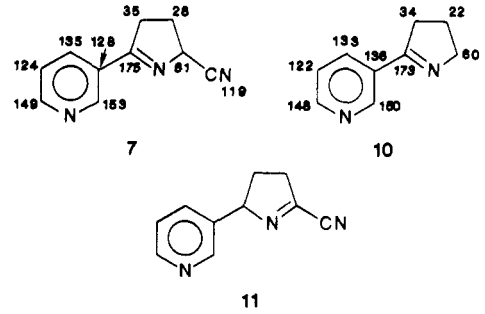
1, R = H
2, R = CO_2H

Exploration of this concept required synthesis of labeled and unlabeled **2** for metabolism studies and bioassays in experimental animals. One previous synthesis of **2** has been reported, but this was not suitable for preparation of the labeled compound.[5,6] In this paper we describe a new synthesis of **2** and its application to the preparation of [*carboxy*-^{14}C]**2**.

Results and Discussion

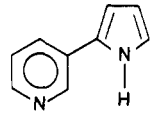
The synthesis is summarized in Scheme I. Reaction of potassium nicotinate (**3**) with the Grignard reagent **4** provided the keto acetal **5** in 52% yield. Use of lithium nicotinate or sodium nicotinate did not have a significant effect on the yield. Hydrolysis of **5** afforded the crude keto aldehyde **6** in 95% yield. This method for preparation of **6** appears to be more convenient than those previously described.[7-9] The keto aldehyde **6** is useful as an intermediate in the synthesis of nornicotine derivatives and is important because it is one of the primary metabolites produced by α-hydroxylation of the potent carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.[2,7]

Reaction of **6** with ammonium cyanide, generated in situ, yielded 5′-cyanomyosmine (**7**) in 70% yield. The structure of **7** was established by its proton NMR spectrum, which showed a triplet of triplets at 5.05 ppm assigned to the 5′-H, and by its ^{13}C NMR spectrum, which was similar to that of myosmine (**10**), as shown below. The ^{13}C NMR data exclude the isomer **11**.



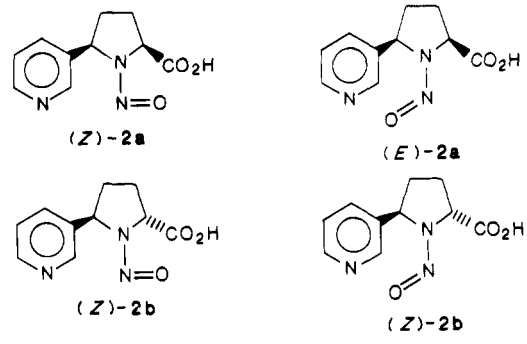
Hydrolysis of **7** produced the unsaturated acid **8**. The position of the double bond in **8** was confirmed by proton NMR. Reduction of **8** gave 5′-carboxynornicotine (**9**). The 400-MHz proton NMR spectrum of **9** indicated that the reduction was stereospecific (see below).

Alternative schemes involving reduction of **7** by $NaBH_4$ or H_2,Pd/C failed, yielding only starting material. The lack of reactivity of **7** with $NaBH_4$ was surprising in view of the facile reduction of **10** to nornicotine under these conditions.[10] Treatment of **7** with $NaBH_3CN$ at pH 3 produced 2-(3-pyridyl)pyrrole (**12**) by dehydrocyanation.



12

Nitrosation of **9** gave **2** in 62% yield. Although **2** has been prepared previously, its NMR spectral properties and configuration have not been examined. The diastereomers **2a,b** can each exist as a pair of *E* and *Z* rotamers depending on the orientation of the nitroso group.[11] The



400-MHz proton NMR spectra and COSY spectra of **2** and **1** were compared. The data for **2** are consistent with a mixture of *Z* (53%) and *E* (47%) rotamers of only one of the diastereomers, either *cis*-**2a** or *trans*-**2b**. On the basis of literature precedent,[12-14] the addition of H_2 to the double bond of **8** is expected to proceed on the face of the molecule opposite from the carboxy group, giving the cis diastereomer **2a**.

An interesting feature of the 400-MHz ^{1}H NMR spectra of **1** and **2** was the resolution of the pyridyl ring protons of the *E* and *Z* rotamers. The pyridyl protons syn to the

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NN=O group resonated at higher field than the anti protons. This is consistent with previous studies.[11] It is also noteworthy that the pyridyl 4-proton of (*Z*)-**2a** (8.84 ppm) was further downfield than the 6-proton of (*Z*)-**2a** (8.78 ppm). This downfield shift of the pyridyl 4-proton was not observed when the spectra were run in Me_2SO nor was it seen in any of the other compounds examined. It probably results from a transannular interaction of the carboxy group and pyridine ring of (*Z*)-**2a**.

The synthesis in Scheme I was readily adapted for the preparation of [*carboxy*-^{14}C]**4**. Conversion of keto aldehyde **6** to 5′-([^{14}C]cyano)myosmine proceeded in 38% yield based on cyanide. Hydrolysis followed by HPLC purification gave [*carboxy*-^{14}C]**8** in 67% yield. Reduction yielded [*carboxy*-^{14}C]**9** in 60% yield after HPLC purification, and nitrosation followed by purification gave [*carboxy*-^{14}C]**2** in 40% yield.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Jeol Model FX90Q and, at Hunter College, City University of New York, Jeol JNM-JX400 spectrometers. They are reported as parts per million downfield from Me_4Si or 3-(trimethylsilyl)-propanesulfonic acid sodium salt as internal standards. ^{13}C NMR spectra were recorded on a Jeol Model FX90 Q spectrometer. IR spectra were recorded on a Perkin-Elmer Model 267 grating infrared spectrophotometer. MS were run with Hewlett-Packard Model 5982A dual-source and 5988A dual-source instruments. High-resolution MS were determined on a VG 70-250 double-focusing magnetic sector instrument, at the Rockefeller University Mass Spectrometric Biotechnology Resource. Thin-layer chromatography (TLC) was performed on silica gel 60 F-254. HPLC was carried out with a Waters Associates System consisting of Model 510 pumps, a Model 660 solvent programmer, and a Model 440 detector. Scintillation counting was performed with a Beckman LS-9000 liquid scintillation system. THF was dried over LAH prior to use. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

1-(1,3-Dioxolan-2-yl)-3-oxo-3-(3-pyridyl)propane (5). A solution of **4** prepared from 3.16 g (0.13 mol) of Mg and 24.4 g (0.135 mol) of 2-(2-bromoethyl)-1,3-dioxolane (Aldrich Chemical Co.) in 100 mL of freshly distilled THF was added dropwise with stirring to a suspension of 16.1 g (0.1 mol) of potassium nicotinate (**3**) in 30 mL of THF at room temperature. A 2-ft double-ended needle was used for the addition. After 20 h the solvent was removed under reduced pressure, and 200 mL of 10% NH_4Cl solution was added. Extraction of the reaction mixture with CH_2Cl_2, washing, drying, and evaporation of solvent left an oil, which upon bulb-to-bulb distillation yielded 10.7 g (52%) of keto acetal **5** as a pale yellow liquid: bp 145–150 °C (0.2 Torr); IR (neat) 1690, 1590 cm^{-1}; NMR ($CDCl_3$) δ 9.2 (d, 1, pyr 2-H), 8.75 (dd, 1, pyr 6-H), 8.23 (ddd, 1, pyr 4-H), 7.40 (dd, 1, pyr 5-H), 5.01 (t, 1, dioxolane 2-H), 3.75–4.05 (m, 4, dioxolane 3,4-H), 3.15 (t, 2, 3-H), 2.15 (dt, 2, 2-H); MS, *m/e* (relative intensity) 147 (6), 108 (3), 106 (22), 87 (4), 86 (42), 79 (5), 78 (24), 73 (100).

4-Oxo-4-(3-pyridyl)butanal (6). A vigorously stirred mixture of 1.85 g (8.9 mmol) of acetal **5** and 10 mL of aqueous 0.2 M oxalic acid was heated at 90 °C for 2 h. It was then cooled, made alkaline with 10% aqueous $NaHCO_3$, and extracted several times with Et_2O. Drying and evaporation of solvent afforded 1.43 g (95%) of keto aldehyde **6** as a pale yellow liquid, which solidified upon cooling to 4 °C. This compound was used in the next step without further purification: IR 1715, 1690 cm^{-1}; NMR ($CDCl_3$) δ 9.90 (s, 1 CHO), 9.20 (br s, 1, pyr 2-H), 8.80 (d, 1, pyr 6-H), 8.25 (ddd, 1, pyr 4-H), 7.45 (dd, 1, pyr 5-H), 3.35 (t, 2, pyr COCH_2), 2.95 (t, 2, CH_2CHO); MS, *m/e* (relative intensity) 135 (57), 134 (39), 121 (33), 107 (10), 106 (100), 79 (8), 78 (65), 77 (5), 73 (6), 51 (12).

5′-Cyanomyosmine (7). To 30 mL of an ice-cold saturated solution of NH_3 in MeOH was added 1.43 g (8.7 mmol) of the keto aldehyde **6**, and the mixture was allowed to stand at 4 °C for 30 min. In the meantime, solutions of 0.6 g of KCN in 5 mL of H_2O and 0.6 g of NH_4Cl in 5 mL of H_2O were prepared and mixed just prior to use. The keto aldehyde–NH_3 and –NH_4CN solutions were then mixed. The mixture was allowed to stand at room temperature for 24 h. The excess NH_3 and solvent were removed under reduced pressure, and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was washed with brine. After drying and concentration, the dark-colored residue was purified by silica gel chromatography (1/99 MeOH/$CHCl_3$) to give 1.05 g (70%) of 5′-cyanomyosmine (**7**) as pale yellow crystals: mp 69–71 °C; IR (KBr) 2235 cm^{-1}; NMR ($CDCl_3$) δ 9.00 (d, 1, pyr 2-H), 8.72 (dd, 1, pyr 6-H), 8.25 (ddd, 1, pyr 4-H), 7.35 (dd, 1, pyr 5-H), 5.05 (tt, 1, 5′-H), 3.15 (m, 2, 3′-H), 2.45 (m, 2, 4′-H); MS, *m/e* (relative intensity) 171 (M^+, 85), 144 (24), 143 (100). Anal. Calcd for $C_{10}H_9N_3$: C, 70.16; H, 5.30; N, 24.54. Found: C, 70.14; H, 5.27; N, 24.57.

2′,3′-Dehydro-5′-carboxynornicotine (8). A vigorously stirred mixture of 1.85 g (10.8 mmol) of 5′-cyanomyosmine (**7**) and 20 mL of 10% aqueous KOH was heated at reflux for 24 h. It was then cooled in an ice bath. The pH was adjusted to 4.0–4.2, and the gel was filtered. The solvent was removed under reduced pressure, isopropyl alcohol (5 mL) was added, and the residue was filtered. Upon cooling of the filtrate, a pale yellow precipitate was formed; 1.32 g (69%); mp 163–166 °C (lit.[5] mp 167 °C); NMR (CD_3OD) δ 9.00 (br s, 1, pyr 2-H), 8.65 (dd, 1, pyr 6-H), 8.35 (ddd, 1, pyr 4-H), 7.55 (dd, 1, pyr 5-H), 4.9 (m, partially obscured by H_2O, HC=CN), 3.30 (t, 1, 5′-H), 2.1–2.6 (m, 2, 4′-H); MS, *m/e* (relative intensity) 190 (40, M^+), 146 (100), 123 (63), 117 (48), 78 (35).

5′-Carboxynornicotine (9). A mixture of 1.1 g (5.8 mmol) of 2′,3′-dehydro-5′-carboxynornicotine (**8**) and 0.5 g of 10% Pd/C in 40 mL of 90% EtOH was stirred under H_2 (1 atm) for 2 h. The catalyst was collected by filtration through a Celite pad, and the filtrate was concentrated in vacuo. The residue was dissolved in a small amount of H_2O, and the amino acid was precipitated by addition of absolute EtOH (6 mL) and cooling (4 °C) overnight: 0.75 g (67%); mp 228–231 °C (lit.[5] mp 206–209 °C); NMR (D_2O) δ 8.65 (br s, 1, pyr 2-H), 8.55 (br s, 1, pyr 6-H), 8.05 (ddd, 1, pyr 4-H), 7.55 (dd, 1, pyr 5-H), 4.85 (t, 1, 2′-H), 4.35 (dd, 1, 5′-H), 2.5 (m, 3), 2.2 (m, 1); MS, *m/e* (relative intensity) 192 (1, M^+), 147 (100), 130 (36), 118 (24), 106 (17).

***N*′-Nitroso-5′-carboxynornicotine (2a).** To a solution of 1.06 g (5.5 mmol) of 5′-carboxynornicotine (**9**) in 30 mL of 50% aqueous acetic acid at 4 °C was added, in portions, 0.65 g (9.42 mmol) of $NaNO_2$. The mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure at 25 °C. The residue was dissolved in 7 mL of 10% aqueous $NaHCO_3$ (pH 6) and upon cooling at 4 °C overnight gave 0.75 g (62%) of **2a** as white crystals: mp 172–175 °C; NMR (400 MHz, D_2O) [*Z* conformer (53%)] δ 9.15 (s, 1, pyr 2-H), 8.84 (d, J = 8.06 Hz, 1, pyr 4-H), 8.78 (d, J = 5.86 Hz, 1, pyr 6-H), 8.07 (dd, J = 8.06, 5.86 Hz, 1, pyr 5-H), 6.00 (dd, J = 8.06, 7.32 Hz, 1, 2′-H), 4.72 (dd, J = 8.80, 4.32 Hz, 2, 5′-H), 2.74 (m, 1, 3′-H), 2.44 (m, 2, 4′-H), 2.36 (m, 1, 3′-H), [*E* conformer (47%)] δ 8.89 (s, 1, pyr 2-H), 8.69 (d, J = 5.86 Hz, 1, pyr 6-H), 8.63 (d, J = 8.06 Hz, 1, pyr 4-H), 8.03 (dd, J = 8.06, 5.86 Hz, 1, pyr 5-H), 5.44 (m, 3, 2′-H and 5′-H), 2.68 (m, 1, 3′-H), 2.51 (m, 1, 4′-H), 2.24 (m, 1, 4′-H), 2.08 (m, 1, 3′-H) [assignments were made in part by comparison to the spectrum of **1**, *Z* conformer, δ 8.44 (d, J = 5.13 Hz, 1, pyr 6-H), 8.35 (s, 1, pyr 2-H), 7.61 (d, J = 8.06 Hz, 1, pyr 4-H), 7.43 (dd, J = 8.06, 5.13 Hz, 1, pyr 5-H), 5.32 (dd, J = 7.32, 7.33 Hz, 1, 2′-H), 4.65 (ddd, J = 6.59, 5.86, 6.60 Hz, 1, 5′-H), 4.54 (ddd, J = 7.33, 5.86, 6.60 Hz, 1, 5′-H), 2.58 (m, 1, 3′-H), 2.27–2.07 (m, 2, 4′-H), 2.01 (m, 1, 3′-H), *E* conformer, δ 8.53–8.52 (s + d, 2, pyr 2-H, pyr 6-H), 7.82 (d, J = 8.06 Hz, 1, pyr 4-H), 7.51 (dd, J = 8.06, 5.13 Hz, 1, pyr 5-H), 5.69 (dd, J = 6.59, 7.33 Hz, 1, 2′-H), 3.96 (ddd, J = 8.06, 8.79, 9.53 Hz, 1, 5′-H), 3.81 (ddd, J = 6.60, 7.33, 8.06 Hz, 1, 5′-H), 2.62 (m, 1, 3′-H), 2.27–2.07 (m, 3, 3′-H and 4′-H)]; MS, *m/e* (relative intensity) (ammonium chloride added) 221 (2, M^+), 191 (7), 148 (20), 130 (21), 118 (100), 105 (55), 91 (24); HRMS (negative chemical ionization), calcd for (M – H) $C_{10}H_{11}N_3O_3$ 220.07214, found 220.07222.

5′-([^{14}C]Cyano)myosmine. To 1.2 mL of an ice-cold saturated solution of NH_3 in MeOH was added 45 mg (0.28 mmol) of freshly prepared keto aldehyde **6**. The mixture was allowed to stand at 4 °C for 30 min. In the meantime, a solution of K^{14}CN (Amersham Corp., 1.9 mCi, 9 mCi/mmol) in 0.12 mL of H_2O and 16 mg of NH_4Cl in 0.03 mL of H_2O were prepared and mixed just prior to use. The keto aldehyde-NH_3 and –NH_4CN solutions were then

mixed. The mixture was allowed to stand at room temperature for 24 h. The excess NH_3 and solvent were removed under reduced pressure, and the residue was partitioned between H_2O and CH_2Cl_2. The organic layer was washed with brine. After drying and concentration, the residue was purified by silica gel chromatography (0–1% MeOH/CH_2Cl_2) to give 14 mg (82 μmol, 738 μCi, 39%) of 5′-([^{14}C]cyano)myosmine. Its purity was determined by HPLC using an ASI Pak-C_{18} column (30 cm × 9 mm) eluted with 5% MeOH/H_2O (flow rate, 1 mL/min; 22 min) and was found to be >95%.

2′,3′-Dehydro-5′-([^{14}C]carboxy)nornicotine. A mixture of 14 mg (82 μmol, 738 μCi) of ([^{14}C]cyano)myosmine and 1.3 mL of 10% aqueous KOH was heated at 90 °C for 18 h. It was cooled in an ice bath, the pH was adjusted to 4–4.2 using 1 N HCl, and the gel was filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by HPLC using a Magnum 9, ODS-3 column (Whatman) eluted with 5% MeOH/H_2O (flow rate, 4 mL/min; t_R 13 min) to give 10.5 mg (55 μmol, 495 μCi, 67%) of 2′,3′-dehydro-5′-([^{14}C]carboxy)nornicotine. Its identity was determined by coinjection with 8 on HPLC and its purity >95%.

5′-([^{14}C]Carboxy)nornicotine. A mixture of 10.5 mg (55 μmol, 495 μCi) of 2′,3′-dehydro-5′-([^{14}C]carboxy)nornicotine and 10 mg of 10% Pd/C in 6 mL of 90% EtOH was stirred under H_2 (1.5 atm) for 2 h. The catalyst was collected by filtration through a Celite pad, and the filtrate was concentrated in vacuo. It was purified by HPLC using a Magnum 9, ODS-3 column eluted with 5% MeOH/H_2O (flow rate, 4 mL/min; t_R 25 min) to give 6.4 mg (33 μmol, 298 μCi, 60%) of 5′-([^{14}C]carboxy)nornicotine, with a retention time identical with that of **9**. It was >95% pure.

***N′*-Nitroso-5′-([^{14}C]carboxy)nornicotine.** To a solution of 6.4 mg (33 μmol, 298 μCi) of 5′-([^{14}C]carboxy)nornicotine in 0.6 mL of 50% aqueous acetic acid at 4 °C was added, in portions, 12 mg (174 μmol) of $NaNO_2$. The mixture was stirred at room temperature for 18 h. The pH was adjusted to 5.5, and the solvent was removed under reduced pressure. The residue was purified by HPLC using a Magnum 9, ODS-3 column eluted with 5% MeOH/H_2O (flow rate, 4 mL/min; t_R 10 min) to give 3.1 mg (13.3 μmol, 120 μCi, 40%, 9 mCi/mmol) of *N′*-nitroso-5′-([^{14}C]-carboxy)nornicotine, with a retention time identical with that of **2**. It was >99% pure.

Kinetic Resolutions of Aliphatic Alcohols with a Fungal Lipase from *Mucor miehei*†

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Received February 23, 1987

A lipase preparation derived from the fungus *Mucor miehei* was employed recently to obtain (*R*)- and (*S*)-2-octanol by kinetic resolution through hydrolysis of the racemic octanoate ester and by esterification of the racemic alcohol with octanoic acid.[1] Of several commercially available lipases evaluated, only the lipase from this fungus exhibited a strong stereobias, thus making it useful for obtaining methyl-*n*-alkylcarbinols in high configurational purity. Moreover, of several *M. miehei* lipases, the preparations of NOVO Company (Lipozyme and NOVO-225) were of greatest value for obtaining these alcohols. a number of resolutions of alcohols (esters) catalyzed by esterases and lipases have been reported,[2] and the utility of enzymatic reactions conducted in organic solvents has been demonstrated.[3] However, esterases are not as readily available as are lipases, and the latter (excepting possibly that of *Candida rugosa*[2] have not been proved useful for the apparently difficult task of resolving straight chain methylcarbinols. Chiral secondary aliphatic alcohols have been the target of asymmetric reduction studies as well, employing chiral reagents[4] and oxidoreductases.[5] Recent research with chiral organoboranes and borohydrides emphasizes the interest in developing methods for such alcohols and the difficulty in achieving the goal.[6,7] The scope of the lipase-catalyzed resolution by esterification using NOVO's lipase from *M. miehei* is reported here.

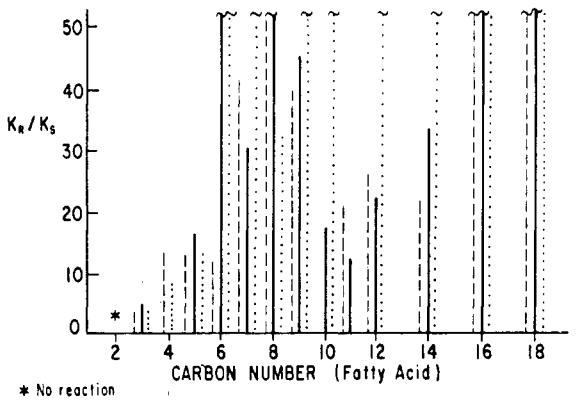

Figure 1. Esterification of 2-hexanol (- - -), 2-octanol (—), and 2-decanol (...) with *n*-alkanoic acids catalyzed by Lipozyme. Reactions were conducted at 30 °C in hexane (see Experimental Section).

Table I. Esterification with Hexanoic Acid[a]

alcohol	E_R	alcohol	E_R
3-methyl-2-butanol	2.1	2-octanol	>50
2-pentanol	2.1	2-decanol	7.7
2-hexanol	9.5	2-dodecanol	14.6

[a] See Experimental Section.

Esterifications were conducted in hexane employing Lipozyme, a formulation of this lipase on an ion exchange resin.[8] The preparation is granular; reaction mixtures may be filtered, and the recovered resin may be used repeatedly. Analyses of reactions were performed by (1) free fatty acid determination to obtain mole fraction conversion (*C*) and (2) conversion of recovered alcohol to diastereomeric carbamates using (*S*)-α-methylbenzyl isocyanate followed by GLC analysis of obtain a value for enantiomeric excess of residual starting material expressed as a fraction (ee). These values may be employed to obtain the enantiomeric ratio (*E*) that is the ratio of the specificity constants (V/K) and is useful in gauging the relative reaction velocities of reacting enantiomers as long as (1) steady-state kinetics apply, (2) the reaction is irreversible, and (3) product inhibition of enzyme is absent.[9] The reaction conditions

† This manuscript is dedicated to Professor George Buchi, Camille Dreyfus Professor of Chemistry, Massachusetts Institute of Technology, on the occasion of his 65th birthday.

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